PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	Rajagopalan et al.
Serial No	10/776,840
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Examiner	Haq
Group Art Unit	1641
Confirmation No.	3029
Title	**METHODS AND COMPOSITIONS FOR DUAL PHOTOTHERAPY**
Atty. Docket	MRD 62DV

Cincinnati OH 45202

May 2, 2007

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

DECLARATION OF RAGHAVAN RAJAGOPALAN, Ph.D.
PURSUANT TO 37 CFR §1.132

I, RAGHAVAN RAJAGOPALAN, declare as follows:

1. I hold a Ph.D. in Organic Chemistry from Columbia University. I have 24 years of experience in the synthesis and use of compounds for medical diagnosis and therapy, which is the subject of this application. I have read the outstanding Office Action of January 3, 2007, and understand the position of the Examiner.

2. I respectfully disagree with the Examiner that claim 38 is indefinite for its recitation of azide, for at least the following reasons.

3. The presence of azide in a cell, upon photoactivation of the azide, results in cell death. When cell viability was determined for cell under control conditions (no light and no azide, light and no azide, no light and azide), cell viability remained high. Only in cells where azide was present and cells were exposed to light did cell death occur.

4. The following conditions were used: The compound E-L-DYE-X-N_3 where E = H, L = single bond, DYE = tetrafluorophenyl, and X = single bond, was administered as the source of azide. The light source was a B-100SP High Intensity Lamp (UVP). Mouse Lewis Lung Carcinoma (LLC) cells were used; cells at a concentration of 0.5×10^6 cells/mL were plated in standard T-25 cell culture flasks in either tissue culture medium or phosphate buffered saline (PBS).

5. The following test parameters were used:

CONTROL/TEST	LIGHT EXPOSURE	PRESENCE OF AZIDE (N_3)
control	- hν	- N_3
control	+ hν (15 min exposure)	- N_3
control	- hν	+ N_3 (1 mM final concentration azide; 30 min incubation)
test	+ hν (5 min exposure)	+ N_3 (1 mM final concentration azide; 30 min incubation)
test	+ hν (15 min exposure)	+ N_3 (1 mM final concentration azide; 30 min incubation)

6. Cells were incubated with the compound for the stated times prior to light exposure. Cells were maintained at 37°C throughout the experiment. Once exposure was complete, cells viability was assessed by manual cell counting in a hemacytometer of Trypan blue stained cells in Hank's Balanced Salt Solution (HBSS). Viable cells were unstained. Non-viable cells were stained. Percent viability was determined as follows:

$$\% \text{ Viability} = \frac{\text{\# Viable Cells Counted}}{\text{Total \# Cells Counted}} \times 100$$

7. The following results were obtained:

Viability of cells exposed to the control experimental conditions, in either media or PBS, was about 90% (no light, no azide; light, no azide; no light, azide). Viability of cells exposed to the test conditions, in either media or PBS, was about 74% for each of the test flasks (light exposure for 5 min and azide; light exposure for 15 min and azide).

8. The data are shown graphically in attached Tables 1 (cells in media) and 2 (cells in PBS).

9. In my opinion, these data clearly demonstrate that azides, upon photoactivation, produce reactive nitrene intermediates that causes cell damage and death, and that, in my opinion, azides are useful for phototherapeutic purposes.

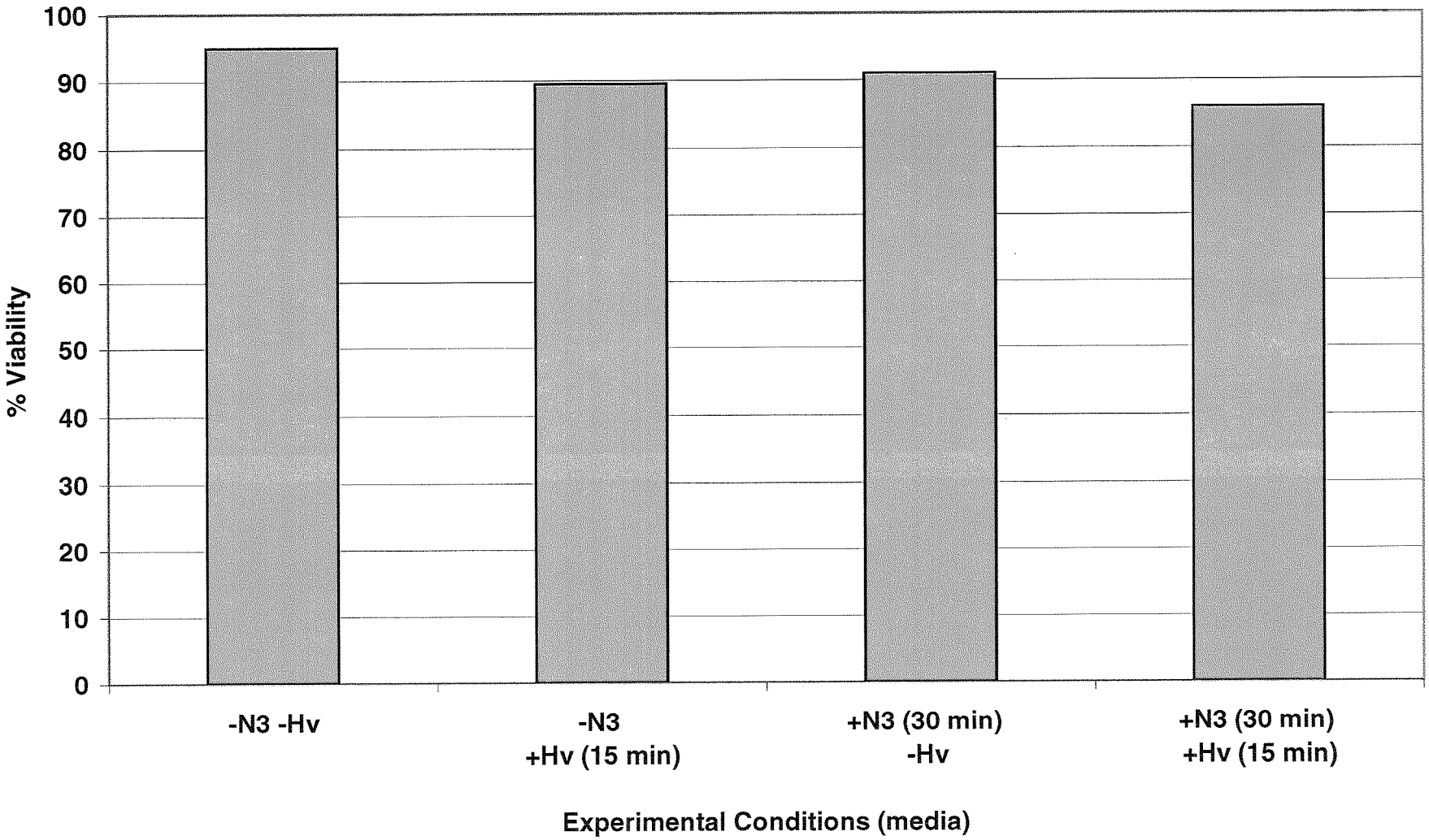
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the subject application or any patent issued thereon.

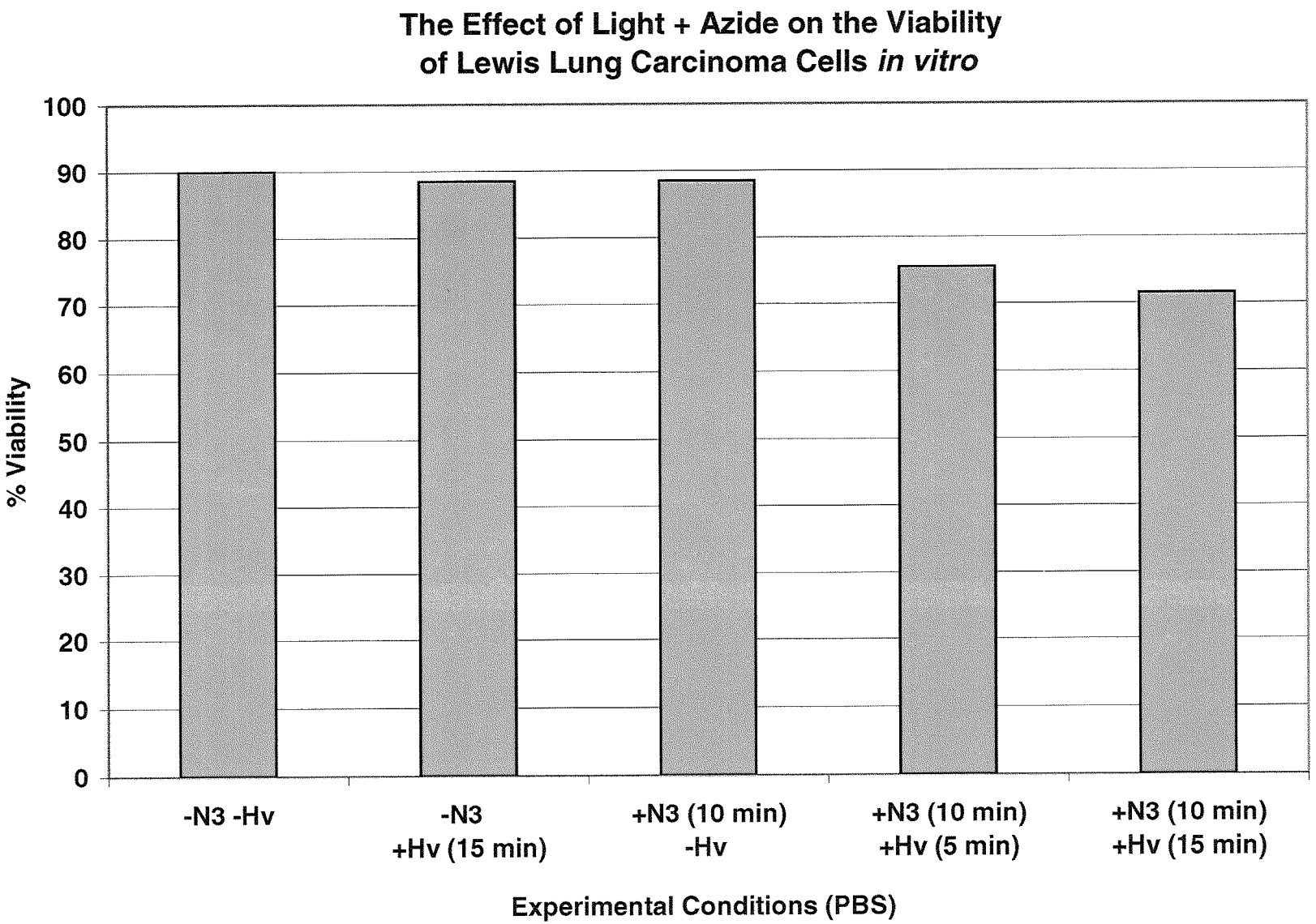
Date: May 2, 2007

Raghavan Rajagopalan

Raghavan Rajagopalan, Ph.D.

The Effect of Light + Azide on the Viability of Lewis Lung Carcinoma Cells *in vitro*




The Effect of Light + Azide on the Viability
of Lewis Lung Carcinoma Cells *in vitro*
% Viability
100
90
80
70
60
50
40
30
20
10
0
-N3 -Hv
-N3
+Hv (15 min)
+N3 (10 min)
-Hv
+N3 (10 min)
+Hv (5 min)
+N3 (10 min)
+Hv (15 min)
Experimental Conditions (PBS)